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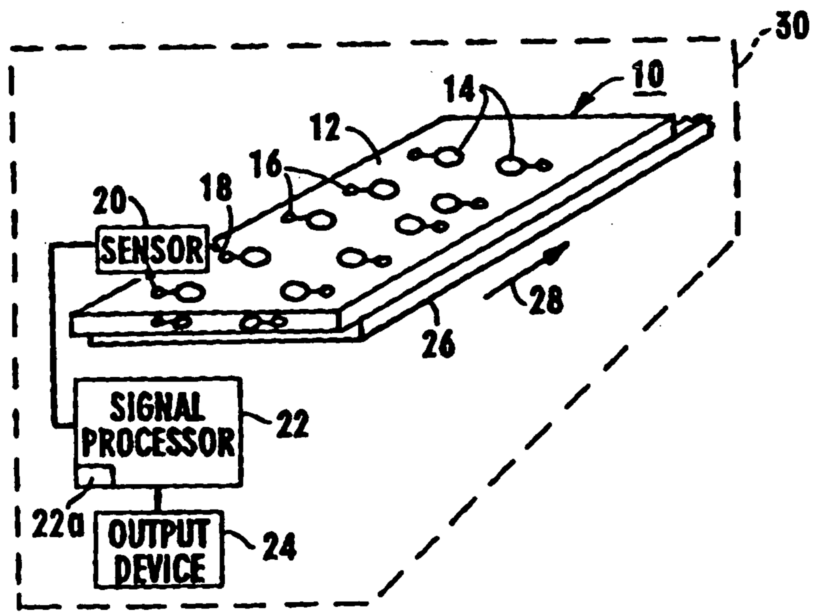
(57) Abstract

A biochemical assay device for detecting the presence of DNA, RNA, proteins, or antibodies (14) includes a colloidal compound having a magnetic marker constituent (16) which binds to the DNA, RNA, protein, or antibody (14) sought to be detected. The DNA, RNA, protein, or antibody (14) with magnetic marker (16) is then juxtaposed with a magnetic sensor (20), and the sensor (20) generates a detection signal representative of the presence of the DNA, RNA, protein, or antibody (14). The detection signal can be correlated to a quantity f DNA, RNA, protein, or antibody (14). A method for detecting the presence of DNA, RNA, proteins, or antibodies (14) using principles of magnetism is also disclosed.



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METHOD AND APPARATUS FOR MAGNETICALLY DETECTING PROTEINS AND NUCLEIC ACIDS

FIELD OF THE INVENTION

The present invention relates generally to biochemical assays, and more particularly to non-radioactive assay techniques for detecting the presence of nucleic acids and proteins.

BACKGROUND

Biochemical assays, performed both on extracts and in situ, for determining the presence and concentration of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), protein, and antibodies in tissue, cells, including E. Coli cells, or serum samples have wide application. For instance, by simply detecting whether a particular sample contains DNA, it can be ascertained whether a DNA amplification procedure that has been performed on the sample (e.g., polymerase chain reaction) has been successful. Also, by determining the quantity of DNA in a particular sample, many attributes of the sample, as well as the effectiveness of whatever laboratory procedure the sample may have undergone, can be usefully ascertained.

Not surprisingly, many biochemical assay techniques have been introduced for analyzing the presence and/or amount of nucleic acid or protein in a sample. Existing techniques include binding a chemiluminescent, fluoriscent, phosphorescent, or radioactive marker, or a colorimetric marker, to the nucleic acid or protein in the sample sought to be assayed, and then employing analytical techniques corresponding to the particular type of assay.

An example of existing nucleic acid/protein assay techniques is disclosed in European Patent application serial no. 85200854.9 to Jannsen Pharmaceutica of Belgium ("the Jannsen application"). In the Jannsen application, a process is disclosed for staining proteins and nucleic acids in a sample with colloidal metal particles, which bind to the nucleic acids/proteins. The metal particles generate a color signal that can be analyzed using spectrophotometry to measure the amount of nucleic acid/protein in the sample being assayed.

A device that uses colorimetric principles is sold by Invitrogen of San Diego, California under the trade name "DNA DipStick™" ("the Invitrogen technology"). In accordance with the Invitrogen technology, a sample substance which contains DNA is deposited on a membrane made of, e.g., nylon, other polymer, or nitrocellulose, and allowed to dry. Then, the membrane is dipped into a colloid solution that contains suspended iron-chloride ($FeCl_2$), causing the $FeCl_2$ to bond with the DNA molecules. Then, a developing agent which includes potassium ferrocyanide is added, causing the color of the $FeCl_2$ (and, hence, the membrane with DNA) to change. Next, the membrane is dried and colorimetrically analyzed to assay the concentration of DNA in the sample.

Unfortunately, each of the above-mentioned assay techniques has certain drawbacks. For example, techniques that rely on spectrophotometry, fluorescence, photoluminescence, or radiography

may not permit a sample to be accurately reanalyzed subsequent to the initial assay. Stated differently, assay products which have been treated with existing reagents based on principles such as chemiluminescence tend to have relatively short shelf lives.

Additionally, to assay a sample using spectrophotometric, photoluminescence, or radiographic techniques, it is frequently necessary to transport a sample to a test facility to perform the assay, preventing immediate and convenient testing of the sample within the facility that prepared the sample. Moreover, radiographic techniques require the use of potentially hazardous radioactive materials. Further, colorimetric techniques that depend on a person to visually correlate an assay color to a DNA concentration, and these techniques thus rely somewhat on human judgement, consequently introducing unwanted, if small, assay errors.

As recognized by the present invention, however, it is possible to assay a sample for nucleic acid, protein, or antibody concentration, while avoiding the drawbacks noted above with previous assay techniques. Accordingly, it is an object of the present invention to provide a method and apparatus for non-radioactively assaying a sample for nucleic acid, protein, or antibody concentration. Another object of the present invention is to provide a method and apparatus for determining the concentration of nucleic acids, proteins, or antibodies in a sample which is repeatable, i.e., which uses a reagent having a relatively long shelf life, and which can be easily performed. Still another object of the present invention is to provide a method and apparatus for determining the concentration of nucleic acids, proteins, or antibodies in a sample which is easy to use and cost-effective.

SUMMARY OF THE INVENTION

A biochemical assay apparatus includes a substrate and a substance which is deposited on the substrate, with the substance including a constituent in the group consisting of: nucleic acids, proteins, and antibodies. A marker is bound to the constituent, and the marker generates a magnetic signal. A sensor senses the magnetic signal of the marker and generates a detection signal in response thereto to indicate the presence of the constituent. Preferably, the detection signal is representative of the quantity of the constituent on the substrate. To this end, a correlator can be provided for correlating the detection signal to a quantity of the constituent.

As intended by the present invention, the marker is magnetic, and can be ferromagnetic, ferrimagnetic, paramagnetic, or superparamagnetic. In the preferred embodiment, the marker includes a plurality of colloidal iron particles, each of which defines a respective magnetic moment, and the magnetic moments of the particles are substantially aligned with each other. As envisioned by the preferred embodiment, the constituent is deoxyribonucleic acid (DNA), the marker is colloidal iron, the sensor is a magnetic field sensor, and the substrate is a nylon matrix.

In another aspect of the present invention, an assay device for detecting the presence of nucleic acids, proteins, and antibodies includes a magnetically marked substance in the group consisting of nucleic acids, proteins, and antibodies. The apparatus further includes a magnetic sensor which is

positioned adjacent the substance for generating a detection signal representative of the presence of the substance.

In yet another aspect of the present invention, a method is disclosed for detecting the presence of a substance in the group consisting of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), protein, and antibodies. The method of the present invention includes binding a magnetic material to the substance, with the magnetic material having a magnetic property. Additionally, the method includes sensing the magnetic property to thereby determine the presence of the substance.

In still another aspect of the present invention, an apparatus for assaying the presence of a magnetically labelled biomolecule in a sample suspected of containing the biomolecule includes a support for the sample. Also, the apparatus includes means for sensing the magnetic label and producing a signal in relation thereto, the sensing means being proximate the support. Further, the apparatus includes output means in communication with the sensing means for presenting the signal produced when the magnetically labelled biomolecule is present in the sample.

In another aspect of the present invention, a method is disclosed for detecting the presence of a biomolecule in a sample suspected of containing the biomolecule. The method includes combining the sample with a colloidal iron reagent to form an admixture thereof, and then maintaining the admixture under predetermined reaction conditions to form an iron-biomolecule complex. Next, the iron-biomolecule complex is separated from any excess iron reagent, and then the iron-biomolecule complex is magnetically sensed to detect the presence of the biomolecule.

The details of the present invention, both as to its structure and operation, can best be understood in reference to the accompanying drawings, in which like reference numerals refer to like parts, and in which:

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a perspective view of a DNA-impregnated substrate juxtaposed with a magnetic sensor, with the sensor and supporting signal processing components shown schematically and the detector housing shown in phantom; and

Figure 2 is a flow chart of the method of the present invention for magnetically detecting DNA.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Referring initially to Figure 1, a support or substrate, generally designated 10, is shown upon which has been deposited a substance 12 by means disclosed below. In one presently preferred embodiment, the substrate 10 is made of a polymeric material, and is preferably a thin, parallelepiped-shaped nylon membrane of the type marketed by Invitrogen of San Diego, California under the trade name "DNA DipStick™". It is to be understood, however, that other substrate materials, i.e., supports, may be used, e.g., porous webs, nitrocellulose, silica, agarouse gels, or polyacrylamide gels, which are appropriate for holding the substances disclosed herein.

In accordance with the present invention, the substance 12 is a biological substance that includes at least one constituent element 14 in the group consisting of deoxyribonucleic acid (DNA) molecules, ribonucleic acid (RNA) molecules, protein molecules, and antibody molecules. Thus, the substance 12 could be derived from any body fluid, e.g., cerebral-spinal fluid, or from other tissue samples or cells, e.g., E. Coli cells, for which it is desired to ascertain the concentration, in the body fluid, of DNA, RNA, proteins, or antibodies. Additionally, the substance 12 could be a substance processed by, e.g., polymerase chain reaction (PCR) amplification techniques, for which it is desired to ascertain the concentration of DNA in the substance after PCR amplification.

As can be appreciated in reference to Figure 1, a respective magnetic marker 16 is chemically bound to each constituent element 14 by means disclosed below to establish a magnetically labelled biomolecule. In one presently preferred embodiment, each magnetic marker 16 is a charged colloidal ferrous chloride ($FeCl_2$) molecule which binds to the associated constituent element 14. It will accordingly be appreciated by those skilled in the art that the number of markers 16 on the substrate 12 is generally linearly proportional to the number of constituent elements 14 on the substrate 12 and, hence, is generally linearly proportional to the concentration of the constituent elements 14 in the substance 12. As intended by the present invention, the term "colloidal ferrous chloride" is meant to include dispersions of particles, e.g., sols, consisting of a metal, metal compound or nuclei coated with a metal or metal compound.

It is to be understood that while the disclosure above refers to a marker 16 that is ferrous chloride and consequently possesses a magnetic property (i.e., magnetism), the marker 16 can include other constituents that exhibit magnetic properties such as ferromagnetism, ferrimagnetism, paramagnetism, or superparamagnetism. For example, the marker 16 can be a ferromagnetic marker in the group including iron, cobalt, nickel, ferrous oxide, ferrous hydroxide and other ferrous alloys, and rare earth elements with atomic numbers between sixty four and sixty nine (64-69), inclusive. Or, the marker 16 can be a ferrimagnetic marker in the group consisting of magnetite (Fe_3O_4), maghemite (Fe_2O_3), and other mixed oxides. Thus, the marker 16 can be made of a ferromagnetic or ferrimagnetic material, the magnetic property of which is the generation of a magnetic field. Alternatively, the marker 16 can be made of a paramagnetic or superparamagnetic material, i.e., a material the magnetic property of which is the tendency to attract a magnet, such as the superparamagnetic material that is disclosed by Vassiliou et al. in J. Appl. Physics 73 (10), 15 May 1993, page 5109. Stated differently, the marker of the present invention generates a magnetic signal, and as intended by the present invention, this magnetic signal can be, e.g., the magnetic field generated by ferromagnetic and ferrimagnetic materials, or the attraction for magnets characteristic of paramagnetic and superparamagnetic materials.

When the marker 16 is magnetic, each marker 16 generates a magnetic moment, represented in Figure 1 by field lines 18. As can appreciated in reference to Figure 1, in the presently preferred

embodiment the magnetic moments of the markers 16 are aligned, i.e., the field lines 18 are generally parallel to each other.

Accordingly, the markers 16 together generate a magnetic field that is representative of the concentration of the constituent element 14 in the substance 12. Consequently, the substrate 10 can be juxtaposed with a magnetic sensor 20 and moved past the magnetic sensor 20, causing the magnetic field of the markers 16 to variably permeate the sensor 20 and thereby cause the sensor 20 to generate a detection signal in response thereto to indicate the presence of the constituent element 14.

The magnetic sensor 20 can be any magnetic sensor that is suitable, when the substrate 10 is moved next to it, for generating a detection signal having a sensitivity that is appropriate for the particular application of the present invention, e.g., mere detection of DNA on the substrate 10 or measurement of the concentration of DNA on the substance 12. For example, when it is desired to simply detect the presence of the markers 16 on the substrate 10 (and, hence, whether the substance 12 contains any constituent element 14), the sensor 20 can be an inductive read head, e.g., the read head used in a Toshiba model KT-53 stereo cassette.

Alternatively, when a relatively precise measurement of the strength of the magnetic field generated by the markers 16 is desired, to ascertain not simply the presence of the constituent element 14 in the substance 12 but the concentration of the constituent element 14 in the substance 12 as well, the sensor 20 can be a magnetoresistive (MR) read head, such as the read heads used in certain existing disk drives and/or the MR heads made by IBM of Armonk New York or Eastman Kodak Co. of Rochester New York and disclosed by Smith et al. in Jour. of App. Physics 69(8), 15 April 1991, page 5082. When the sensor 20 is an MR head that is embedded in a chip, the chip can be formed with a channel and the substance 12 deposited in the channel for assaying. Or, the sensor 20 can be a magnetic force microscope, SQUID sensor, metal film Hall-effect device, or a ultra-high sensitivity susceptometer (for sensing paramagnetic and superparamagnetic markers) such as the device disclosed by Slade et al. in IEEE TRANSACTIONS ON MAGNETICS, vol. 23, no.5, September, 1992, page 3132.

As shown in Figure 1, the sensor 20 is electrically connected to a signal processor 22 that receives the detection signal and generates a signal representative of the concentration of the constituent element 14, e.g., DNA in the substance 12 in response thereto. It is to be understood that the signal processor 22 includes signal processing circuitry known in the art for processing signals from magnetic sensors, as well as a correlator 22a for generating a DNA concentration signal based upon the detection signal from the magnetic sensor 20. The correlator 22a can be a programmable chip or a microprocessor.

As intended by the present invention, when the substrate 10 is a thin membrane as shown, the correlator 22a correlates the detection signal with a concentration of the constituent element 14 in the substance 12, with the correlation being linearly dependent on the strength of the detection signal. Then, the correlator 22a generates a DNA concentration signal in response which is representative of the

concentration of the constituent element 14 in the substance 12. Apart from the type of substrate used, it is to be understood that the correlator 22a can be calibrated to generate accurate DNA concentration signals by means well-known in the art, e.g., by passing several substrates having known quantities of DNA deposited thereon next to the sensor 20 and correlating the resulting detection signals to the known concentrations.

If desired, the signal from the signal processor 22 can be sent to an output device 24. In accordance with the present invention, the output device 24 can be any suitable device, e.g., headphones, audio-visual computer display, or analog meter, which generates a sensory indication of the detection signal.

Still referring to Figure 1, if desired, a transporter 26 can be provided, and the substrate 10 can be positioned on the transporter 26 to move the substrate 10 past the sensor 20 in the direction of the arrow 28. Alternatively, the sensor 20 can be moved past the substrate 10 in the direction of the arrow 28 and in a direction normal thereto, in a raster-scan type motion, to generate a two-dimensional data output, e.g., an image, having an "x" dimension and a "y" dimension. Further, the two-dimensional data output can be transformed into a three-dimensional output wherein the third dimension ("z" dimension) represents magnetic signal intensity. The entire combination of structure disclosed above can be disposed in a housing 30.

Now referring to Figure 2, one preferred embodiment of the present invention, for which DNA is the constituent element 14, can be seen. Starting at block 32, the DNA sample is prepared as desired by means well-known in the art. More particularly, as one example the substance 12 can be disposed in a gel, or a slab gel, or a capillary gel, and the constituent element 14, i.e., DNA in the embodiment shown, can then be separated in the gel by, e.g., electrophoresis. Then, the gel in which the substance 12 is disposed can function as a substrate, or the gelatinized substance 12 deposited onto a membrane.

Next, when a nylon membrane is to be used as the substrate, the substance 12 with constituent element 14, e.g., DNA, is deposited onto the substrate 10 by means well-known in the art. For example, when using the DNA DipStick™ mentioned above as the substrate 10, about one microliter (1.0 μl) of substance 12 is deposited onto the substrate 10. Then, at block 36, the substance 12 is allowed to dry for about five to fifteen minutes. Alternatively, the substance 12 can be UV cross-linked to the substrate 10.

At block 38, the substrate 10 is disposed in a wash solution for about ten seconds. A suitable wash solution can contain about 0.1 normal hydrochloric acid (HCl), and can be provided by Invitrogen of San Diego, California as part of Invitrogen's "DNA DipStick™ kit".

At block 40, the substrate 10 is disposed in a colloidal iron coupling solution for about three minutes, to thereby bond the markers 16 with the constituent element 14 (DNA). Stated differently, at block 40 the substrate 10 is disposed in a colloidal iron reagent to form an admixture of the reagent and magnetically labelled constituent element 14 (DNA). Like the wash solution, the colloidal iron coupling

solution can be procured from Invitrogen of San Diego, California. In addition to ferrous chloride, the Invitrogen coupling solution contains cacodylic acid.

Next, at block 42, the substrate 10 is disposed in distilled water for about twenty seconds to separate any excess iron reagent from the substance 12, and then, at block 44, the substrate 10 preferably is disposed in a developing solution for about three minutes, to enhance the magnetic signal. In one presently preferred embodiment, the developing solution includes potassium ferrocyanide, and can be procured from Invitrogen.

After disposing the substrate 10 in the developing solution, the substrate 10 is disposed in a wash solution at block 46 for about twenty seconds. Then, at block 48, the substrate 10 is dried.

At block 50, the magnetic moments of the markers 16 are aligned with each other. This can be accomplished naturally by the Earth's magnetic field, but is more preferably accomplished by juxtaposing the substrate 10 with a strong permanent magnet.

Then, at block 52, the substrate 10 is juxtaposed with the magnetic sensor 20 and moved relative to the sensor 20 to cause the sensor 20 to generate the detection signal. Preferably, the substrate 10 is closely juxtaposed with the sensor 20. More preferably, the substrate 10 is distanced from the sensor 20 by only a few microns or less, in order to improve the sensitivity of the present invention. At block 54, the correlator 22a receives the detection signal from the sensor 20 and correlates it to a concentration of the constituent element 14 in the substance 12.

The above-described steps were performed using a substrate 10 which was a DNA DipStick™ that had been colorimetrically assayed according to the Invitrogen DNA DipStick™ Instruction Manual, available from Invitrogen and incorporated herein by reference. After colorimetric assay, the substrate 10 having DNA deposited thereon was moved past a Toshiba model KT-53 stereo cassette read head magnetic sensor 20. The output device 24 was a set of headphones, and an audible signal was heard on the headphones when the portions of the substrate 10 that had been colorimetrically indicated as containing DNA were moved past the magnetic sensor 20. The concentration of DNA that was magnetically sensed was colorimetrically assayed at one half nanograms per microliter (0.5ng/μl).

It is to be understood that the present invention fully contemplates other methods for combining the substrate 10 with substance 12, constituent element 14, and marker 16. For example, when the constituent element 14 is DNA, the marker 16 can be bound to the DNA during electrophoretic separation of the DNA in a gel substrate. Or, the DNA can be disposed in a gel, the gel deposited on a membrane substrate, and then the gel removed from the substrate, leaving behind the DNA. Alternative methods of combining the substrate 10 with substance 12, constituent element 14, and marker 16 may become apparent and known in the art, and used in conjunction with the principles disclosed herein, without departing from the scope of the appended claims.

While the particular method and apparatus for magnetically detecting proteins and nucleic acids as herein shown and described in detail is fully capable of attaining the above-described objects of the

invention, it is to be understood that it is the presently preferred embodiment of the present invention and is thus representative of the subject matter which is broadly contemplated by the present invention, that the scope of the present invention fully encompasses other embodiments which may become obvious to those skilled in the art, and that the scope of the present invention is accordingly to be limited by nothing other than the appended claims.

WHAT IS CLAIMED IS:

1. A biochemical assay apparatus, comprising:

a substrate;

a substance deposited on the substrate, the substance including a constituent in the group consisting of: nucleic acids, proteins, and antibodies;

a marker bound to the constituent, the marker generating a magnetic signal; and

a sensor for sensing the magnetic signal of the marker and for generating a detection signal in response thereto to indicate the presence of the constituent.

2. The apparatus of Claim 1, wherein the detection signal is representative of the quantity of the constituent on the substrate.

3. The apparatus of Claim 1, wherein the marker is magnetic.

4. The apparatus of Claim 3, wherein the marker is ferromagnetic or ferrimagnetic.

5. The apparatus of Claim 3, wherein the marker is paramagnetic or superparamagnetic.

6. The apparatus of Claim 1, wherein the marker includes a plurality of colloidal iron particles, each particle defining a respective magnetic moment, the magnetic moments of the particles being substantially aligned with each other.

7. The apparatus of Claim 1, wherein the constituent is deoxyribonucleic acid (DNA), the marker is colloidal iron, the sensor is a magnetic field sensor, and the substrate is a nylon matrix.

8. The apparatus of Claim 1, further comprising a correlator for correlating the detection signal to a quantity of the constituent.

9. An assay device for detecting the presence of nucleic acids, proteins, and antibodies, comprising:

a magnetically marked substance in the group consisting of nucleic acids, proteins, and antibodies; and

a magnetic sensor positioned adjacent the substance for generating a detection signal representative of the presence of the substance.

10. The assay device of Claim 9, further comprising a substrate on which the substance is deposited, and a marker for bonding to the substance, thereby magnetically marking the substance, the marker including a plurality of colloidal iron particles, each particle defining a respective magnetic moment, the magnetic moments of the particles being substantially aligned with each other.

11. The assay device of Claim 10, wherein the substance includes deoxyribonucleic acid (DNA), the sensor is a magnetic field sensor, and the substrate is a polymer matrix.

12. The assay device of Claim 10, further comprising a correlator for correlating the detection signal to a quantity of the substance.

13. A method for detecting the presence of a substance in the group consisting of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), protein, and antibodies, comprising the steps of:

binding a magnetic material to the substance, the magnetic material having a magnetic property; and

sensing the magnetic property to thereby determine the presence of the substance.

14. The method of Claim 13, further comprising the steps of:

providing a substrate and a magnetic sensor;

depositing the substance on the substrate;

providing a colloid including the magnetic material and disposing the substrate with substance in the colloid to cause the magnetic material to bond with the substance; and

juxtaposing the substrate with the magnetic sensor for causing the magnetic sensor to generate a detection signal representative of the presence of the substance.

15. The method of Claim 14, further comprising the step of correlating the detection signal to a quantity of the substance.

16. The method of Claim 15, wherein the magnetic material includes a plurality of magnetic particles, each defining a respective magnetic moment, and the method further comprises the step of juxtaposing the substrate with a magnet to align the magnetic moments prior to juxtaposing the substrate with the magnetic sensor.

17. An apparatus for assaying the presence of a magnetically labelled biomolecule in a sample suspected of containing the biomolecule, the apparatus comprising:

a support for the sample;

means for sensing the magnetic label and producing a signal in relation thereto, the sensing means being proximate the support; and

output means in communication with the sensing means for presenting the signal produced when the magnetically labelled biomolecule is present in the sample.

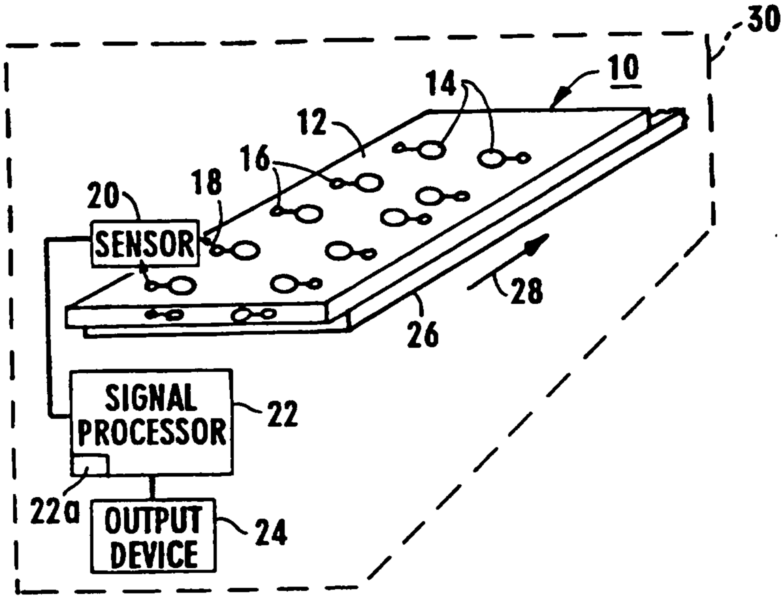
18. A method for detecting the presence of a biomolecule in a sample suspected of containing the biomolecule, comprising the steps of:

combining the sample with a colloidal iron reagent to form an admixture thereof;

maintaining the admixture under predetermined reaction conditions to form an iron-biomolecule complex;

separating the iron-biomolecule complex from any excess iron reagent; and

magnetically sensing the iron-biomolecule complex to detect the presence of the biomolecule.


30
10
14
12
16
18
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SENSOR
28
26
SIGNAL
PROCESSOR
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22a
OUTPUT
DEVICE
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Fig. 1

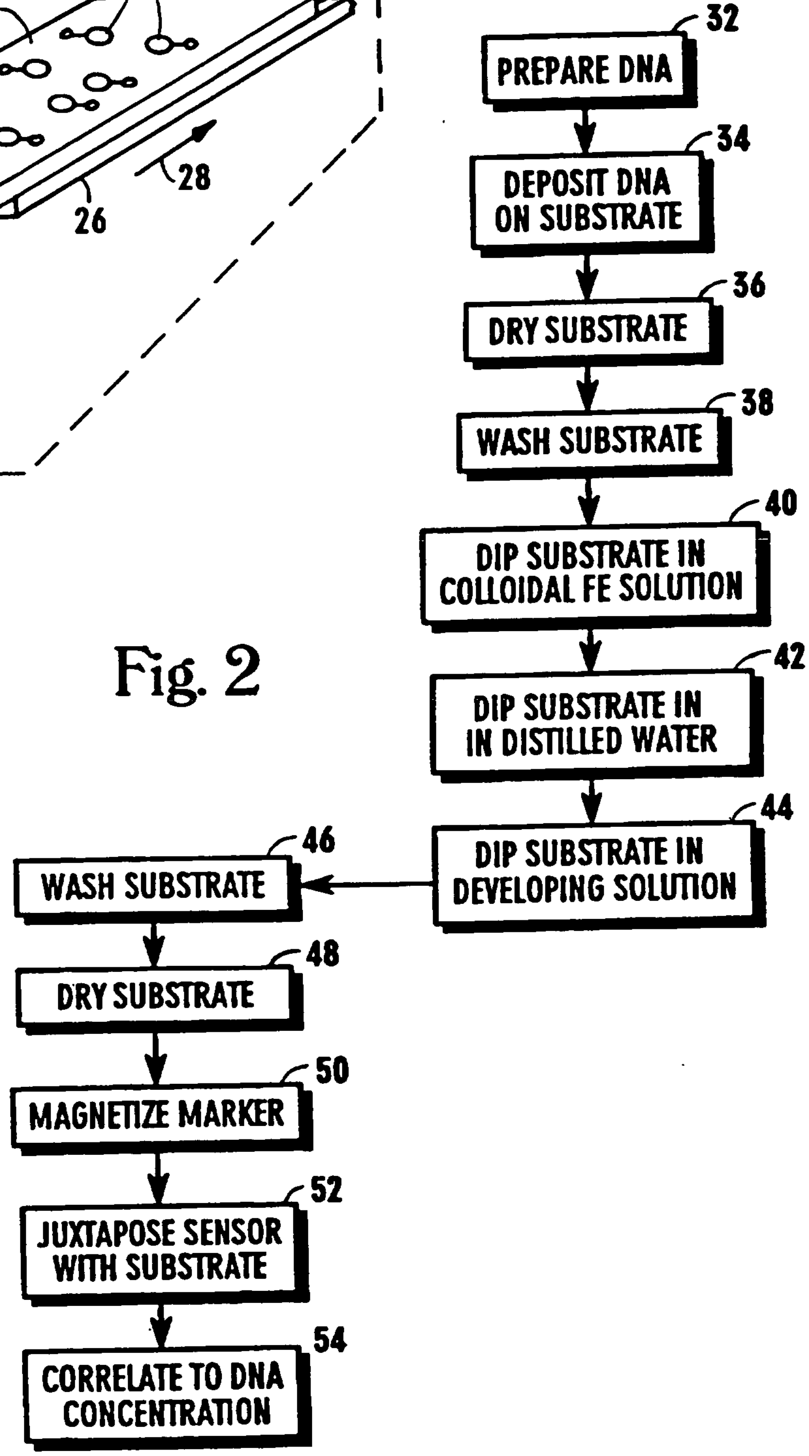
PREPARE DNA
32
DEPOSIT DNA
ON SUBSTRATE
34
DRY SUBSTRATE
36
WASH SUBSTRATE
38
DIP SUBSTRATE IN
COLLOIDAL FE SOLUTION
40
DIP SUBSTRATE IN
IN DISTILLED WATER
42
DIP SUBSTRATE IN
DEVELOPING SOLUTION
44
WASH SUBSTRATE
46
DRY SUBSTRATE
48
MAGNETIZE MARKER
50
JUXTAPOSE SENSOR
WITH SUBSTRATE
52
CORRELATE TO DNA
CONCENTRATION
54

Fig. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/10503

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/68
US CL :435/6
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 422/50; 436/526, 806, 807

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG
search terms: magnetic, sensor or detect?, marker or particle#, metal particle#, stain, Fox, John S., DNA or RNA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	JP, A, 63-302367 (NIPPON TELEGRAPH & TELEPHONE) 09 December 1988, see abstract.	1-3, 8-9, 13, 17 ---------------- 4-7, 10-12, 14-16, 18
Y	EP, A, 0,165,633 (JANSSEN PHARMACEUTICA N.V.) 27 December 1985, see page 1, lines 25-31, page 2, lines 18-35, and page 3, lines 10-17.	6-7, 10-12, 14-16, 18
Y	BIOPHYSICAL JOURNAL, Volume 52, issued October 1987, Valberg et al., "Magnetic Particle Motions within Living Cells, Physical Therapy and Techniques", pages 537-550, see especially abstract, page 537, column 1, and page 538, column 1.	6, 10-12, 16

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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